The Examiner questions whether cultured keratinocytes caused to undergo apoptosis by UV irradiation are appropriate models to use to predict the behavior of hair follicle keratinocytes undergoing apoptosis in the hair follicle as a result of aging or disease. Keratinocytes of the epidermis cannot be readily distinguished from keratinocytes of the hair follicle, as they share morphologic, biochemical and molecular markers. Follicle keratinocytes are known to migrate up to the surface of denuded skin and reconstitute the epidermis. That is, hair follicle keratinocytes can become epidermal keratinocytes when called upon as a result of wounding. Hair follicle keratinocytes are derived from the same cells as epidermal keratinocytes during embryonic development. Hair follicle keratinocytes represent a population of the epidermal keratinocytes that enter into hair follicle-specific differentiation during embryogenesis. Stem cells that are located in the hair follicle bulge are able to differentiate into epidermal and hair follicle keratinocytes (Taylor, G. *et al., Cell 102*:451-461, 2000; copy enclosed as Appendix 1).

Two journal articles were enclosed with the Declaration of Barbara A. Gilchrest, M.D. Under 37 C.F.R. § 1.132 filed on 8 April 2002. The articles present examples of other researchers' use of cell cultures as models of hair follicles. The first, Detmar *et al.* (Detmar, M. *et al., J. Invest. Dermatol. 101(1 Suppl.)*:130S-134S, 1993), described the culturing of keratinocytes and their use in studies on regulation of the hair growth cycle. The second, Moll (Moll, I., *J. Invest. Dermatol. 105(1)*:14-21, 1995), described a study of keratinocytes in which it was found that the keratinocytes grown in culture were capable of differentiation, as required for hair formation. Therefore, keratinocytes grown in culture are an accepted model of phenomena in hair follicles.

It has long been known that hair follicles involute (during catagen, when they cease to produce a hair shaft) by apoptosis of the follicular keratinocytes. It has also been established in the literature that at least one mechanism by which this occurs is binding of neurotrophins [e.g., brain-derived neurotrophic factor ( BDNF)] to the p75 receptor. This is shown, for example, in genetically engineered BDNF overexpressing mice whose hair follicles enter catagen prematurely and whose full grown fur coat is shorter than the fur coat of wildtype mice. See Botchkarev, V.A. *et al.*, *FASEB J. 13*:395-410, 1999; copy enclosed as Appendix 2. The fact that other

factors may also influence hair growth or cell death is irrelevant here. These other factors are constant in the experiments.

NIH 3T3 cells were used in studies on the binding of peptides because they overexpress the p75 receptor that is the target of the nerve growth factor fragments and peptides comprising amino acid sequence KGA (KGA peptides). It can be shown that the peptides work explicitly and exclusively by interacting with this receptor. The peptides have no effect on cells lacking p75. Further, it has been shown that a KGA peptide interferes with $^{125}$I-A-β binding to the p75 receptor in NIH 3T3 cells that overexpress the receptor and that a control peptide differing only in one amino acid (required for p75 receptor binding) does not (Zhai *et al.*, *J Invest. Dermatol. 110*:481, 1998; copy enclosed as Appendix 3). Further, it has been shown that the KGA peptide interferes with A-β-induced p75 receptor aggregation in these cells (Zhai *et al.*, *J. Invest. Dermatol. 112*:548, 1999; copy enclosed as Appendix 4)." The use of a cell type naturally lacking the receptor, but transfected to express a specific receptor, is a well validated means of establishing a specific biologic role for that receptor. All keratinocytes express the p75 receptor. The NIH 3T3 cells served as a convenient model for the binding of peptides to the p75 receptor on keratinocytes.

The Examiner states that the Declaration of Barbara A. Gilchrest, M.D. Under 37 C.F.R. § 1.132 filed on 8 April 2002 demonstrates that the KGA peptide causes a delay in hair follicles reaching later catagen stages...." Also pertinent is the fact that hairs are retained during the catagen period and are shed only after completion of catagen, so that slowing or retarding the catagen phase prolongs the period that each hair remains in place. Thus, retarding the catagen phase causes hairs to be maintained in the hair follicles.

The Examiner states specific objections: "It is unclear how catagen was measured, why anagen was not measured, or if this resulted in hair growth." Catagen stages were determined by well established, universally accepted morphologic criteria (Straile, W.E. *et al.*, *J. Exp. Zoology 148*:205-221, 1961; copy enclosed as Appendix 5). Catagen stage I is by definition identical to anagen stage VI, as stated in Straile *et al.* on page 206, second column. The experiment shown in Figure 1 (page 4) of the Declaration was performed in mice after synchronization of the hair follicles, specifically to examine the impact of the KGA peptide on the early catagen phase, when

neurotrophins acting through the p75 receptor are known to cause the process of hair follicle involution, leading to hair loss.

Respectfully submitted,

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MARKED UP VERSION OF AMENDMENTS

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Replace the paragraph at page 28, line 29 through page 29, line 3 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Substances identified in this method are substances that specifically alter the apoptotic mechanism in melanocytes and [kerantincytes] keratinocytes. For example, substances that mimic nerve growth factor can be tested in an assay such as the one described above to evaluate their activity in inhibiting apoptosis. Additionally, substances identified and evaluated by this method can be peptides, organic molecules, small organic molecules, antibodies or antibody fragments.

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

33. (Amended) A method of maintaining hair growth in a vertebrate comprising [inhibiting p75 nerve growth factor receptor-mediated apoptosis in keratinocytes by] contacting the [keratinocyte] keratinocytes of the vertebrate with a ligand or pseudo-ligand that binds to [a] keratinocyte p75 nerve growth factor [receptor] receptors, thereby inhibiting apoptosis, and [inducing] maintaining hair growth.

34. (Twice Amended) The method of Claim 33 wherein the [pseudo-ligand is nerve growth factor, or] ligand is a biologically active fragment [thereof, wherein the fragment is a] of nerve growth factor [peptide, or the pseudo-ligand is a nerve growth factor pseudo-ligand that binds to the p75 nerve growth factor receptor].

35. (Amended) [A] The method of Claim [34] 33 wherein the pseudo-ligand is a peptide comprising the amino acid sequence lysine-glycine-alanine.

36. (Amended) [A] The method according to Claim 35 wherein the peptide is selected from the group consisting of SEQ ID NO: 4, 9 and 10.

45. (Amended) A method of treating alopecia areata in a [vertebrate] human, said method comprising [maintaining hair growth in the vertebrate comprising inhibiting p75 nerve growth factor receptor-mediated apoptosis in keratinocytes by] contacting [the] keratinocytes in the skin of the human with a ligand or pseudo-ligand of p75 nerve growth factor receptor, in an amount sufficient to inhibit apoptosis, [that binds to a keratinocyte p75 nerve growth factor receptor,] thereby [inhibiting apoptosis, and] maintaining hair growth.

46. (Twice Amended) The method of Claim 45 wherein the [pseudo-ligand] ligand is [nerve growth factor, or] a biologically active fragment of nerve growth factor [thereof, or a nerve growth factor pseudo-ligand that binds to the p75 nerve growth factor receptor].

47. (Amended) [A] The method of Claim [46] 45 wherein the pseudo-ligand is a peptide comprising the amino acid sequence lysine-glycine-alanine.

48. (Amended) [A] The method according to Claim 47 wherein the peptide is selected from the group consisting of SEQ ID NO: 4, 9 and 10.

49. (Twice Amended) A method of treating a [male] human with male pattern baldness, comprising [inhibiting p75 nerve growth factor receptor-mediated apoptosis in keratinocytes by] contacting the keratinocytes in the [male] human with a pseudo-ligand or ligand of p75 nerve growth factor receptor, in an amount sufficient to inhibit apoptosis, [that binds to a keratinocyte p75 nerve growth factor receptor,] thereby inhibiting apoptosis, and maintaining hair growth.

50. (Twice Amended) The method of Claim 49 wherein the [pseudo-ligand] ligand is [nerve growth factor, or] a biologically active fragment [thereof, or a] of nerve growth factor [pseudo-ligand] that binds to the p75 nerve growth factor receptor.

51. (Amended) [A] The method of Claim [50] 49 wherein the pseudo-ligand is a peptide comprising the amino acid sequence lysine-glycine-alanine.

52. (Amended) [A] The method according to Claim 51 wherein the peptide is selected from the group consisting of SEQ ID NO: 4, 9 and 10.